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Application of physiological and biochemical indices for screening and assessment of drought tolerance in durum wheat genotypes

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Abstract

Field experiments were conducted at the Khorasan-e- Razavi Agricultural and Natural Resource Research Center, in Mashhad during the 2007and 2008 season, to determine drought tolerance in promising durum wheat genotypes. The experiments were laid out using a split plot arrangement, in randomized complete block design with three replications. Four irrigation regimes and five wheat genotypes were assigned to the main-plots and sub-plots respectively. Analysis of the data showed that with advancement of the growth and developmental stages from booting to anthesis and soft dough in the different wheat genotypes planted, the values of relative water content, canopy temperature depression and proline accumulation in leaves, decreased under both optimum and water deficit conditions. In addition, water limitation during grain filling significantly decreased relative water content and canopy temperature depression at the soft dough stage. It caused an increase in the proline content by 22%, 47% and 114% in the vegetative, reproductive and grain filling stages respectively, compared to the control. The findings also showed that the highest values for relative water content, canopy temperature depression and proline accumulation at the different growth and developmental stages of the plants under water deficit conditions, belonged to the G2 durum wheat genotype (RASCON_37/BEJAH_7) compared to the other bread and durum wheat genotypes studied. From the results, it can be concluded that this promising genotype is able to maintain high levels of relative water content under water deficit conditions. This study has also shown that the physiological and biochemical indices used to evaluate plant response to water deficit were effective in assessing promising durum wheat genotypes for drought tolerance.

Key word: Canopy temperature depression, durum wheat, proline, relative water content, water deficit.
Abbreviation: Canopy temperature depression (CTD), proline (pro), relative water content (RWC).

Introduction

Drought is one of the major limiting factors affecting plant productivity worldwide and influences almost all aspects of plant biology (Romo et al., 2001; Pan et al., 2002). It is well documented that accessibility of water for plant growth is a key aspect determining plant distribution in natural ecosystems and is the single most important limiting parameter in agricultural ecosystems. An understanding of the genetic and physiological basis of drought tolerance would facilitate the development of improved crop management and breeding techniques and lead to better yield in unfavorable environments (Rekika et al., 1998). Relative water content (RWC) is used extensively to determine the water status of plants relative to their fully turgid condition. According to Beltrano et al., (2006), plants that are able to maintain high levels of RWC under water deficit conditions, are less affected by stress and are able to maintain normal growth and yield. The findings of several researchers working on durum wheat genotypes have shown that with decreasing RWC in the leaves, under water deficit conditions, the water balance of the plants are disrupted (Molnár et al., 2004; Dulai et al., 2006). In addition to this other studies have reported that canopy temperature depression (CTD) is a superior indicator of a genotype's physiological suitability to drought tolerance (Fischer et al., 1998; Rashid et al., 1999; Ayeneh et al., 2002). As a result, it has been used in many practical evaluations of plant response to water deficit. Recently, Balota et al. (2007) reported the application of CTD to estimate crop yield and to rank genotypes for tolerance to drought. Previous reports by Blum et al. (1982) have shown that canopy temperature differences among various wheat and triticale cultivars were lowest and highest under favorable and water deficit conditions, respectively. Furthermore, canopies with higher water content are indicative of genotypes with higher biomass resulting from larger rates of carbon fixation associated with greater stomata conductance and therefore, cooler canopies (Babar et al., 2006). Previously Siddique et al., (2000) had shown that drought stress significantly decreased RWC and this in turn had a pronounced effect on the photosynthetic rate. They suggested that an increase in leaf and canopy temperature, due to drought stress, resulted from an increase in respiration and a decrease in transpiration due to stomata closure. Another common physiological response to drought stress in many plants is the accumulation of the amino acid proline (Mafakheri et al., 2010). It is well documented that accumulated proline plays a role as a compatible solute in plants, regulating and reducing water loss from the cell under water deficit conditions. In addition to this, it has an adaptive role in plant stress tolerance (Verbruggen and Hermans, 2008). Proline has repeatedly been shown to increase under water stress and is potentially an important contributor to osmotic adjustment (Mattioni et al., 1997). In wheat, it has been reported that osmotic adjustment is an important factor explaining differences in genotype yield or yield stability

Table 1. The effect of different irrigation regimes and genotypes on canopy temperature depression, leaf relative water content and proline accumulation at the different growth and developmental stages

Treatments	Canopy Temperature Depression (°C)			Relative Water Content (%)			Proline concentration
	Booting	Anthesis	Soft dough	Booting	Anthesis	Soft dough	(mg g^{-1} DW)
Irrigation regime							
I1	8.4a	5.9a	4.8a	74.3a	70.1a	61.8a	1.49d
I2	7.0b	5.4a	4.1b	69.3b	64.9b	60.9ab	1.81c
I3	4.8c	4.8b	3.2c	58.5c	39.5c	54.1b	2.19b
I4	7.9a	5.6a	1.9d	73.6a	68.7ab	42.8c	3.19a
LSD	0.67	0.43	0.54	4.16	4.59	7.05	3.86
Sx	0.12	0.08	0.10	0.79	0.87	1.34	0.73
Genotype							
G1	6.7bc	5.3bc	2.82c	67.8bc	59.6bc	53.4bc	1.64d
G2	7.4ab	5.8a	4.53a	73.7a	65.6a	57.2a	2.66a
G3	6.9abc	5.4abc	3.78b	70.6ab	62.4ab	57.3a	2.57a
G4	7.6a	5.5ab	3.48b	65.9c	57.8c	55.1ab	1.86c
G5	6.5c	5.1c	2.96c	66.6c	58.4c	51.5c	2.13b
LSD	0.67	0.41	0.43	3.79	3.78	2.87	0.21
Sx	0.17	0.11	0.11	0.98	0.98	0.74	0.05

Column sharing the same letters indicates no significant differences at $p < 0.01$

(Teulat, 1997). Although, it has been suggested that proline (pro) is not directly involved in drought tolerance and is not essential for improved resistance, wherever proline increase does occur, it improves resistance (Errabll et al., 2006). Bayoumi et al., (2008) reported that in wheat, relationship between grain yield and proline accumulation was positively correlated under water deficit condition. It has been suggested that an increase in the proline content might be an adaptation mechanism to overcome stressful conditions and could supply energy for growth and survival, thereby enabling the plant to tolerate stress (Sankar et al., 2007). Although the application of physiological indices has been used in drought screening techniques by previous workers, the assessment of drought tolerance using physiological and biochemical parameters in promising durum wheat genotypes has been relatively scarce. The present study was carried out to identify quantifiable traits and appropriate physiological and biochemical indicators that would facilitate crop improvement for drought tolerance. In addition to this the wheat genotypes studied were ranked for drought tolerance under the different water deficit conditions.

Results and Discussion

Relative water content (RWC)

As shown in Fig.1, with advancement in the growth and developmental stages, from booting to anthesis and finally the soft dough stage, RWC values decreased under the different irrigation regimes (I1, I2, I3 and I4). The highest RWC was observed under optimum irrigation (I1) at the booting, anthesis and soft dough stages, compared to the plants water deficit conditions (I2, I3, and I4). There was a significant difference in RWC values between optimum irrigation (I1) and the I3 and I4 water deficit treatments at the grain filling (soft dough) phase. The largest decrease in RWC at the anthesis stage belonged to the plants grown with water limitation at the reproductive phase (I3), while the largest decrease at the soft dough stage was observed in plants with water limitation at the grain filling stage (I4). Recently Dulai et al., (2006), Adejare and Umebese (2007) reported that RWC values were significantly reduced under water stress treatments at the booting, anthesis and soft dough growth stages in the bread and durum wheat plants they studied. The highest RWC value recorded amongst the various genotypes studied, belonged to the G2 and G3 genotypes at all the growth stages (booting, anthesis and soft dough). However, with the advancement of the growth stages from booting to anthesis and the soft dough stage, the RWC values gradually decreased slightly (Table 1 and Fig. 2) in all the genotypes studied.

Canopy Temperature Depression (CTD)

The decreasing trend in CTD from the booting to the grain filling stage under optimum and water deficit conditions was generally similar to that observed with RWC (Fig. 1 and Fig. 3). The highest CTD values (8.43 ˚C) were seen in plants under the I1 treatment at the booting stage, whilst the lowest (1.95 ˚C) CTD values were seen in plants under the I4 treatment, at the soft dough stage. It was also observed that plants under the I1 and 14 irrigation treatments exhibited high CTD values at the booting stage, which is probably related to their similar irrigation regime up to the anthesis period. Table.1 and Fig. 3 also shows a significant gradual decrease in the CTD values at the soft dough stage where values of 4.8 ˚C, 4.1 ˚C, 3.2 ˚C and 1.9 ˚C were recorded under the I1, I2, I3 and I4 water treatments respectively. A similar decrease in CTD values, with the advancement in growth and developmental stages from booting to anthesis and the soft dough stage was reported by Siddique et al., (2000). As mentioned above, they suggested that leaf and canopy temperature increase under drought stress is probably due to an increase in respiration and a decrease in transpiration as a result of stomatal closure. With regard to the importance of canopy temperature during grain filling, the interaction effects of the irrigation regimes and genotypes on canopy temperature depression at the soft dough stage is shown in Fig. 4. It shows that the highest CTD values belonged to the G2 genotype compared to other genotypes under optimum and water deficit conditions. The Chamran (G3) bread wheat genotype, which is known to be drought tolerant cultivar, exhibited the second highest CTD value after the G2 genotype at the I2 and I4 water deficit treatments.

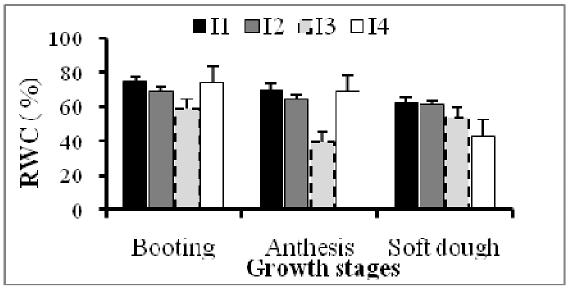

Fig 1. The effect of different irrigation regimes on the leaf relative water content (RWC) at different growth stages. Results are shown as mean ± standard error (p<0.01), obtained from three replicates

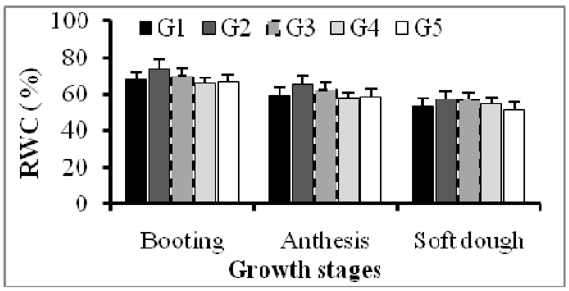

Fig 2. Relative water content (RWC) in different genotypes at different growth and developmental stages. Results are shown as mean ± standard error (p<0.01), obtained from three replicates

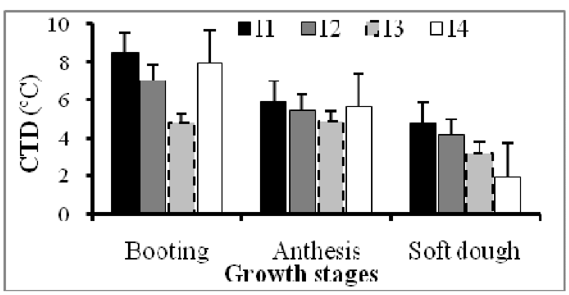

Fig 3. The effect of different irrigation regime on canopy temperature depression in durum wheat genotypes at different growth stages. Results are shown as mean ± standard error (p<0.01), obtained from three replicates

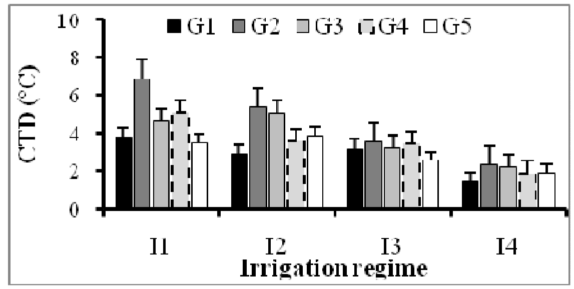

Fig 4. Interaction effects of irrigation regimes and genotypes on canopy temperature depression (CTD) at the soft dough stage. Results are shown as mean ± standard error (p<0.01), obtained from three replicates

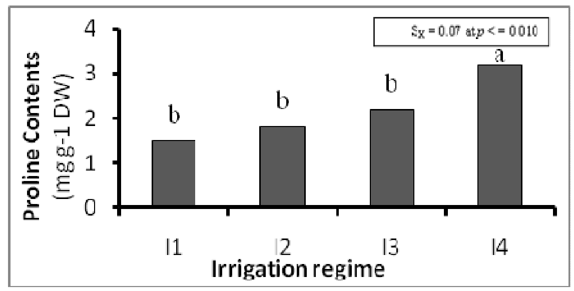

Fig 5. The effect of different irrigation regimes on the total proline content (mg g^{-1} DW) in the leaves of durum wheat.

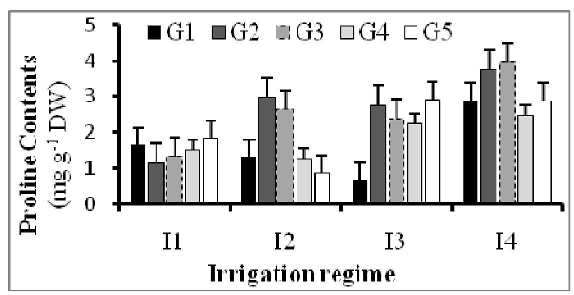

Fig 6. The effect of different irrigation regimes on the total proline content (mg g^{-1} DW) in the leaves of the different durum wheat genotypes

The remarkable physiological response shown by the G2 genotype to CTD makes it a prime candidate for screening as a desirable drought tolerant genotype. Balota et al. (2007) recently suggested that canopy temperature depression is a good indicator to estimate crop yield as well as genotype ranking for drought tolerance.

Proline content

As shown in Table 1, Fig. 5 and Fig. 6, the amount of proline accumulation was dependent on the irrigation at the different stages of plant growth, where water deficit increased the proline content in the leaves by 22%, 47% and 114% of control under the I2, I3 and I4 treatments, respectively. There was a markedly significant difference between the I4 treatment (water deficit during grain filling) and the other irrigation treatments. There were also significant differences in proline concentration amongst the durum and bread wheat genotypes. The highest proline content was observed in the G2 and G3 genotypes, whilst the G5, G4 and G1 exhibited lower values, respectively (Fig. 6). The interaction effects between the different irrigation regimes and genotypes also showed that proline accumulation increased under water deficit conditions compared to optimum irrigation. The highest proline accumulation values were seen in the G3 (3.95 mg g^{-1} DW) and G2 (3.77 mg g^{-1} DW) under I4 water deficit conditions. However, the highest proline content values under optimum irrigation conditions were seen in the G5 (1.8 mg g^{-1} DW) and G1 (1.6 mg g^{-1} DW) durum wheat genotypes. These genotypes also exhibited higher proline values at the vegetative (I2) and reproductive (I3) phases under water deficit condition compared to optimum irrigation (Fig. 6). Bayoumi et al., (2008) reported a similar positive relationship between grain yield and proline accumulation under water deficit conditions in wheat. This suggests that the high proline content in the G2 and G3 genotypes is probably a positive adaptive mechanism for overcoming the water stress conditions. Furthermore, Sankar et al. (2007) reported that high proline accumulation in plants could provide energy for growth and survival and thereby help the plant to tolerate stress. It is now well known that proline accumulation in plant leaf cells, as a compatible solute, plays an important role in regulating water loss from the cells under water deficit and osmotically stressful conditions (Bayoumi et al., 2008). It is therefore reasonable to suggest that the selection of new drought tolerance genotypes based on high proline accumulation, can be advocated as a parameter for selection of stress tolerance, as it can be effective in enhancing drought tolerance in plants (Silverira et al., 2003; Jaleel et al., 2007).

Material and methods

Experimental site and plant material

The field experiment was carried out at the Khorasan-e-Razavi Agricultural and Natural Resource Research Center, Iran. The following four irrigation regimes: I1, optimum irrigation; I2, no irrigation from one-leaf to floral initiation; I3, no irrigation from floral initiation to anthesis; I4, no irrigation after anthesis were the main-plots and four durum-promising genotypes (G1, HAI - OU-17/ GREEN - 38; G2, RASCON - 37/ BEJAH - 7; G4, RASCON - 39 / TILO - 1; G5, GARAVITO3 / RASCON37// GREEN8) and a bread wheat cultivar (G3, Chamran) were the sub-plots.

Sampling and measurements

Relative water content (RWC)

The RWC was determined at booting, anthesis and soft dough growth stages. It was calculated according to the following equation, where FW is the fresh weight, SW is the water-saturated weight and DW is the dry weight (Turner, 1981).

$$RWC = (FW - DW) \times 100 / (SW - DW)$$

Canopy temperature depression (CTD)

The CTD was measured with a handheld infrared thermometer (Model THI-500, TASCO, Japan). The data were taken from the same side of each plot at 1m distance from the edge and approximately 50 cm above the canopy. All canopy temperature measurements were made within 2 h of solar noon, and in a south-facing direction, to minimize sun angle effects, as suggested by Turner et al., (1986).

Proline content

Assessments of proline content were performed in fully expanded leaves according to the method of Pesci and Beffagna (1984). The leaf samples (50 mg) were extracted with 10 ml of sulphosalicylic acid solution (3%) for 1 hour at room temperature and then filtered on Whatman fiberglass paper. A part of the extract (30 mg) was added to 4 ml of ninhydrin and 4 ml of acetic acid and incubated in a boiling water bath for 1 h. After cooling rapidly in ice, 5 ml of toluene was added to the sample and the mixture strongly shaken. The absorbance of the toluene phase, containing the colored complex was measured at 515 nm versus a toluene blank. From the absorbance readings obtained the proline content in each sample was calculated by means of a standard calibration curve, using known amounts of proline.

Statistical analysis

The experimental design was a split plot arrangement based on a complete randomized block design with three replications. One way ANOVA was applied to evaluate the significant difference of the parameters studied in the different treatments. Significant differences among the mean values were compared via the Duncan's Multiple Range Test ($P < 0.05$ and $P < 0.01$).

Conclusion

From this study it can be concluded that the G2 durum wheat genotype (RASCON_37/BEJAH_7) exhibited the highest values of relative water content, canopy temperature depression and proline accumulation at the different growth and developmental stages of the plants, compared to the other bread and durum wheat genotypes studied. This promising genotype was able to maintain high levels of relative water content under limited water conditions and thus stabilize its water balance under moisture stress. This study has also shown that the physiological and biochemical indices used to evaluate plant response to water deficit were effective in assessing promising the durum wheat genotypes for drought tolerance. The remarkable response shown by the G2 genotype makes it a good candidate for cultivation, to ensure high yield is obtained under drought conditions.

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